--45. A complex comprised of at least one negatively charged nucleic acid and at least one positively charged polymeric conjugate with the bond therebetween being electrostatic in nature,

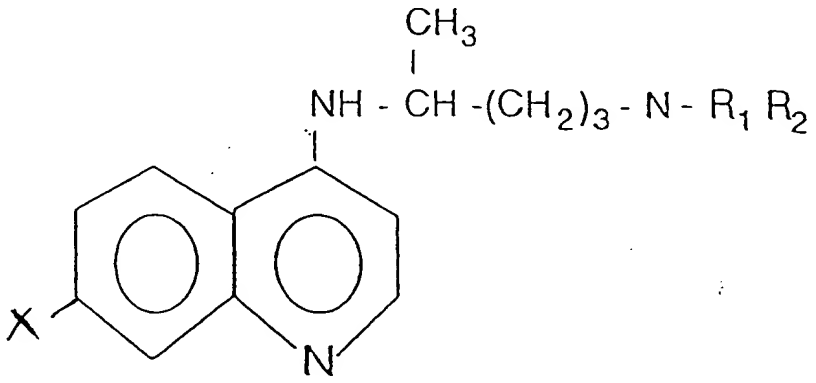
the polymeric conjugate containing a polylysine formed from monomers having free NH_3^+ groups,

at least 10% of free NH_3^+ groups of the said polylysine are substituted by residues which can be protonated in a weakly acid medium causing destabilization of cell membranes,

and optionally at least one some of the free NH_3^+ groups of ~~the~~ said polylysine ~~can be~~ is substituted by a molecule with a recognition signal recognized by a cell membrane receptor,

with the proviso that all the free NH_3^+ groups of the said polylysine make up at least 30% of the number of monomers of the skeleton of the polymeric conjugate,

wherein said residue causing destabilization of cell membrane in a weakly acid medium belong to the family of quinolines of the formula:



in which R_1 is hydrogen, R_2 is $-(CH_2)_n-CO_2-H$, X is hydrogen or chlorine and n is an integer from 1 to 10, wherein said recognition

signal is selected from the group consisting of:

simple osides selected from the group consisting of α or ß conformers of 2-deoxy, of 2-amino or 2-deoxy, 2-acetamido neutral monosaccharides; α or ß conformers of glycuronic acid derivatives of neutral monosaccharides; α or ß conformers of L-iduronic acid, of keto-deoxy-octonic acid, of N-acetyl neuraminic acid, or of N-glycoloyl-neuraminic acid; and α or ß conformers of neutral 6-deoxy monosaccharides;

~~or~~ a disaccharide selected from the group consisting of lactose and mannopyranosylα-6-mannopyranose,

and
~~or~~ complex osides selected from the group consisting of Lewis^a, Lewis^b, Lewis^x, oligomannosides and oligolactosamines or peptides.

The
46. A complex of claim 44 wherein said quinolines are selected from the group consisting of 7-chloro-4-(amino-1-methylbutylamino)-quinoline, N^4-(7-chloro-4-quinolinyl)-1,4-pentanediamine, 8-(4-amino-1-methylbutylamino)-6-methoxyquinoline (pyrimaquine), N^4-(6- methoxy-8-quinolinyl)-1,4-pentanediamine,

to the family of pterines ?

and to the family of pyridine.

47. The complex of claim 45 wherein the free NH_3^+ groups of the polylysine are substituted with a non-charged gluconyl residue causing a reduction in the positive charge of the polymeric conjugate which facilitates salting out of the nucleic acids upon dissociation of the complex.

48. The complex of claim 45 wherein said recognition signal

is a peptide chosen from the group consisting of

(a) anti-inflammatory peptides recognized by receptors of the vascular wall,

(b) ligand peptides of integrins,

(c) chemiotactic factors and

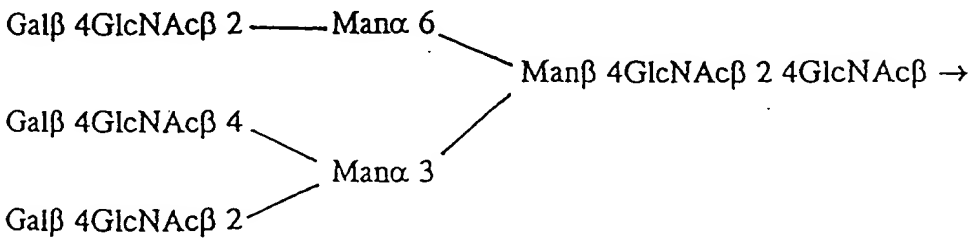
(d) peptide hormones.

49. The complex of claim 45 wherein:

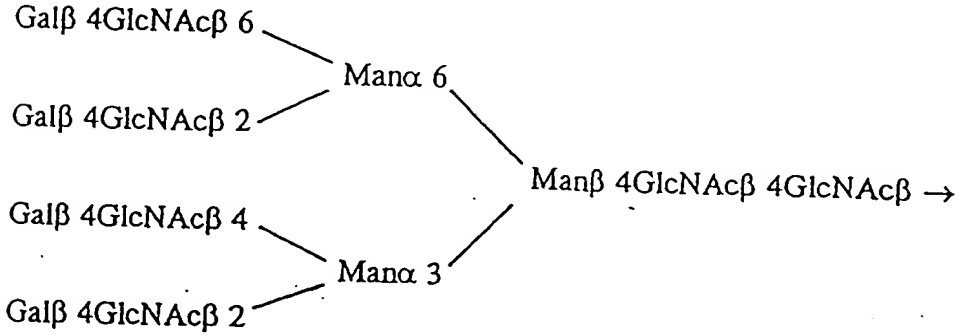
- the monosaccharide is selected from the group consisting of galactose, mannose, fucose, glucose, ribose, xylose and rhamnose and

- the oligosaccharide is selected from the group consisting of

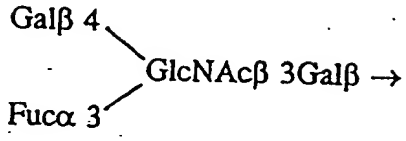
(a) Asialo-oligoside of the type of triantennar lactosamine: asialoglycoprotein receptor



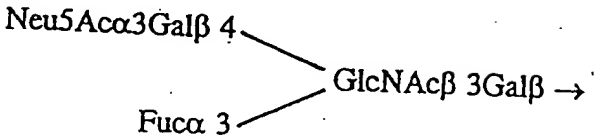
(b) Asialo-oligoside of the type of tetraantennar lactosamine : asialoglycoprotein receptor



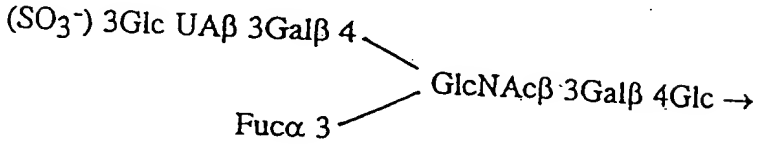
(c) Lewis x : LECAM 2/3



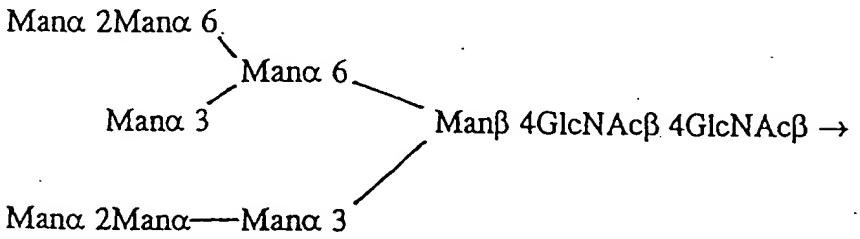
(d) Lewis x sialyl : LECAM 3/2



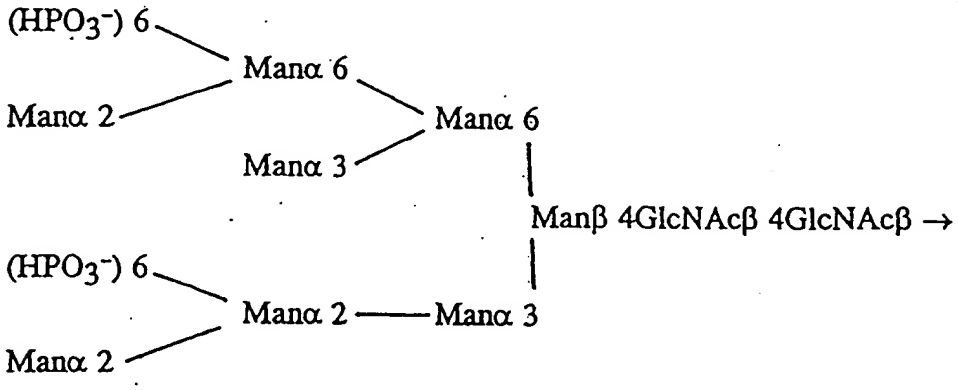
(e) Sulphated Lewis x derivative (HNK1) : LECAM 1



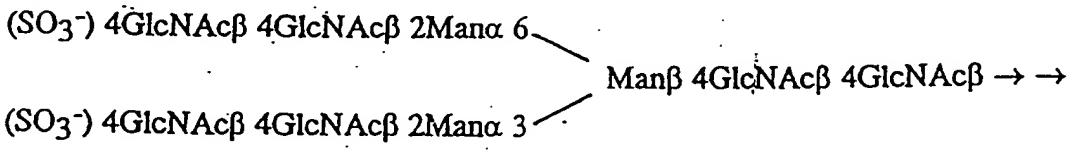
(f) Oligomannoside : mannose receptor



(g) Phosphorylated oligomannoside : mannose 6-phosphate receptor



(h) Oligosaccharide of the type of sulphated lactosamine: sulphated GalNAc 4 receptor:



i. Lactose

j. Fucα2Gakß3(fucα4)GlcNAcß1Galß3Glc

k. Fucα4(Gaß3)GlcNAcß3Galß and

l. Manα6-man.

50. The complex of claim 49 wherein the peptides are selected from the group consisting of

- vasodilator intestinal polypeptide (VIP)

HSDAVFTDNYTRLRKQMAVKKYLNSILN-NH_2

- antrial natriuretic polypeptide (ANP)

SLRRSSCFGGRMDRIGAQSGLGCNSFRY

- lipocortin

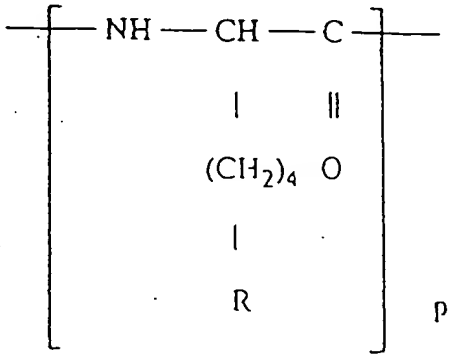
HDMNKVLDL

- bradykinin

RPPGFSPER;

peptides containing the RGC sequence, fibroonectin ligand, formyl peptides and their antagonists, α-MSH FMLP, (N-formyl-Met-Leu-Phe) and Ac-SYMEHFRWGKPV-NH_2.

51. The complex of claim 45 wherein the polymeric conjugate has the formula:



I

wherein:

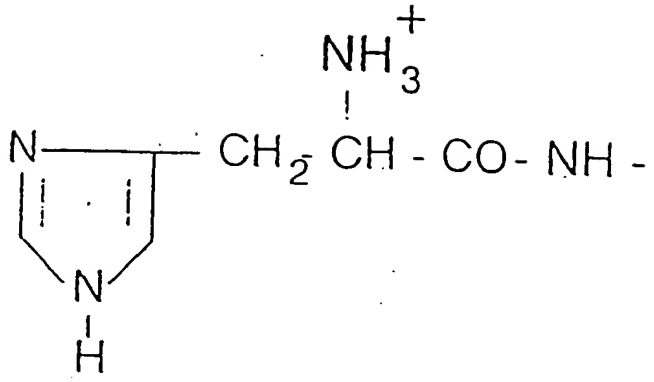
- p is an integer from 15 to 900,

- 10 to 45% of the radical R being a residue with an imidazole nucleus,

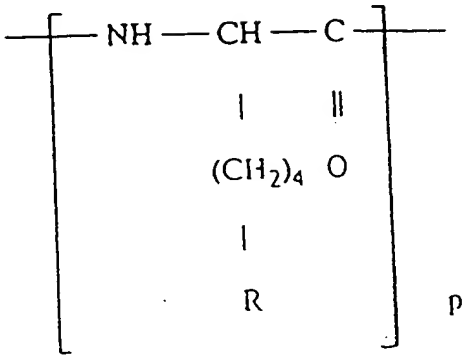
- 10 to 90% of R being free NH_3^+ groups,

- and optionally 0 to 45% of R being -NH-CO-$(CHOH)_m$-R_1, m is an integer from 2 to 15, and R_1 is hydrogen or alkyl of 1 to 15 carbon atoms.

52. The complex of claim 51 wherein R is a residue with an imidazole nucleus of the formula:



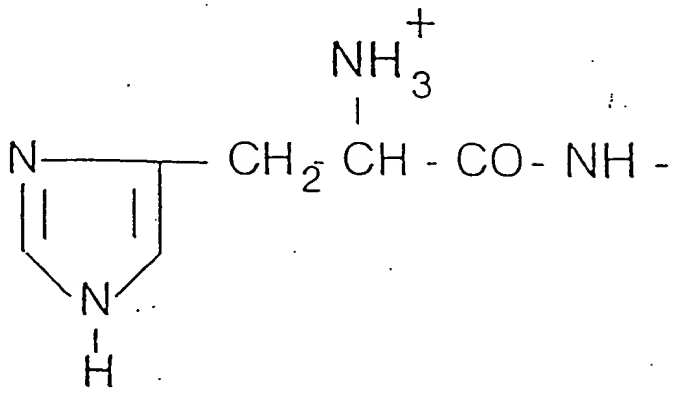
53. The complex of claim 51 wherein the polymeric conjugate has the following formula:

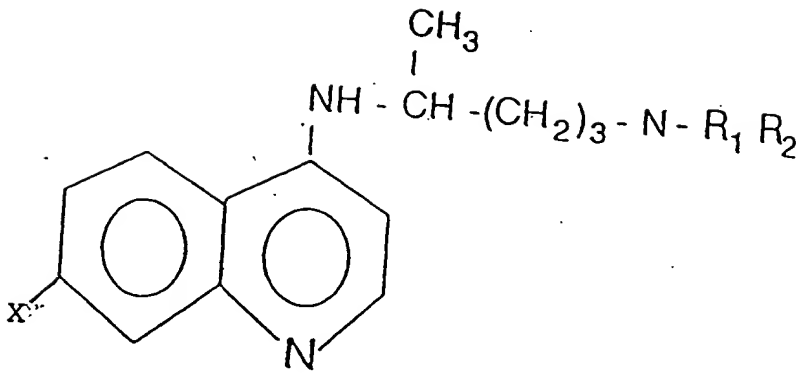


wherein:

- p is an integer from 15 to 900,

- 10% to 45% of R is a residue having an imidazole nucleus and optionally a free NH_3^+, it being possible for R to have the formula:





in which R_1 is hydrogen, R_2 is $(CH_2)_n$-CO_2-H, X is hydrogen or chlorine and n is an integer from 1 to 10, wherein said recognition signal is selected from the group consisting of:

- simple osides selected from the group consisting of α or ß conformers of 2-deoxy, of 2-amino or of 2-deoxy, 2-acetamido neutral monosaccharides; α or ß conformers of glycuronic acid derivatives of neutral monosaccharides; α or ß conformers of L-iduronic acid, of keto-deoxy-octonic acid, of M-acetyl-neuraminic acid, or of N-glycoloyl-neuraminic acid; and α or ß conformers of neutral 6-deoxy monosaccharides;

- a disaccharide selected from the group consisting of lactose and mannopyranosylα-6-mannopyranose,

and complex osides selected from the group consisting of Lewis^a, Lewis^b, Lewis^x, oligomannosides and oligolactosamines, and peptides.

56. The Positively charged polymeric conjugate according to claim 55 wherein the free NH_3^+ groups of the polylysine are substituted with a non-charged residue causing a reduction in the positive charge of the polymeric conjugate which facilitates salting out of the nucleic acids upon dissociation of the complex, the said non-

charged residue being a gluconyl.

57. A composition comprising the complex of claim 45 and an inert pharmaceutical carrier.

58. A method of transfecting cultured cells comprising incubating said cells in the presence of a composition of claim 57 under conditions wherein said composition enters said cells, and the nucleic acid comprised in the complex of said composition is released.

59. The method of claim 58 wherein the cells are selected from the group consisting of

-cells of haematopoietic strains;

-dendritic cells;

-liver cells;

-skeletal muscle cells;

-skin cells;

-fibroblasts,

-keratinocytes,

-dendritic cells,

-melanocytes;

-cells of the vascular walls;

endothelial;

smooth muscle;

-epithelial cells of the respiratory tract;

-cells of the central nervous system;

-cancerous cells; and

-cells of the immune system.

Applicants are submitting herewith an Abstract of the Disclosure on a separate sheet of paper. Applicants have cancelled non-elected claims 41 to 44 but reserve the right to file a divisional application directed thereto. The specification has been amended to insert reference to the PCT application.

The sequence listing required by the Examiner has already been submitted on May 1, 2001.

With respect to the Examiner's rejection of the claims under 35 USC 112, second paragraph, it is deemed that the new set of claims comply with the statute and are free of the objections noted by the Examiner in the office action. Therefore, withdrawal of these grounds of rejection is requested.

Claims 21 to 40 were rejected under 35 USC 103 as being obvious over the French '316 or the Midoux et al patents taken with the Wang et al reference. The Examiner states that D1 and Midoux, the two primary references, describe a complex of at least one negatively charged nucleic acid and at least one positively charged polymeric conjugate bonded by electrostatic interaction. The Examiner states that the polymeric conjugate contains a polymer of monomeric units with free NH_3^+ groups which may be substituted in a ratio of at least 10% by gluconyl based non-charged residues. The Examiner states that the difference between the primary references and the present invention is that the claimed invention

is directed to histidine residues that are protonable in a weakly acidic medium and have a functional group enabling them to be bound to the polymer while not being recognized by a cell membrane receptor.

The Examiner states that the secondary reference describes the fusion-mediating properties of polyhistidine relative to liposomes and the concept diffusion is caused by the poly-cationic nature of polyhistidine having an acid pH and the combination of the polycation with membrane phospholids that induce phase separation in the dual lipid layer can be seen from the Abstract. The Examiner further states that the secondary reference indicates that fusion mediating behavior associated with polyhistidine having a low pH is more effective than the one associated with calcium ions or polylysine and deems that it would have been obvious to incorporate histidine residues to any of the free NH_3^+ groups of the polylysine of D1 to enhance the fusion and translocation.

Applicants respectfully traverse these grounds of rejection since in the present invention, polylysine is partially substituted by residues which can induce destabilization of membranes in an acid medium and particularly, histidyl residues and is efficient in the absence of auxiliary elements such as chloroquine or fusiogenic peptides. With respect to the French '31C or D1, this patent relates to a complex between the negatively charged nucleic acid and at least one positively charged polymeric conjugate with the

binding between the nucleic acid and the polymeric conjugate being of electrostatic nature. The polymeric conjugate contains a polymer formed of monomer units carrying free NH_3^+ functions such that the free NH_3^+ functions of the above mentioned units are substituted at a rate of at least 10% of non-charged residues being responsible for a diminution of positive charges with respect to the same substituted polymeric conjugates. Moreover, the patent relates to the preparation and use of polylysine substituted by gluconoyl residues and possibly, biligands of small molecular weight for gene transfer. This modified polylysine requires the use of auxiliary elements such as chloroquine or fusiogenic peptides in an acid medium to make easier the transmembrane passage of DNA into cytosol after endocytosis in the acid besicles.

With respect to the D3 reference of Wang et al, Applicants will concede that this suggests the protenation of the histidine residues of the viral protein with an acidic pH as an alternative fusion means. However, Applicants have shown that the in-corporation of histidine residues to some of the free e-amino groups of the polylysine allows the permeabilization of the plasma membrane of mammalian cells in weakly acidic medium. This was not described in the D3 document which shows the polyhistidine destabilized phosphatidylserine liposomes. It was not obvious that it would be efficient on the membrane of living cells. One of the unexpected results of Applicants' invention is that the polylysine bearing histidine residues are able to destabilze cell membranes in

acidic medium when it is complexed with a nucleic acid. Moreover, protenated histidines would be expected to interact with the phosphate groups of the nucleic acid such as described for the polymer poly (Lys, His) in the copolymer/DNA complexes as taught by Santella et al, H.J. 1997 entitled Interaction between poly(L-lysine[48], L-histidine[52]) and DNA, Biopolymers, Vol. 16, pp. 1879-1894. Therefore, they would not be expected to interact with cell membranes to induce their destabilization.

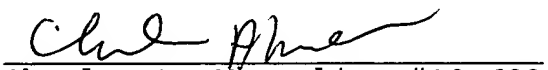
Moreover, the D3 document relates to a histidine polymer which is a linear polymer. In Applicants' invention, histidine is grafted onto a linear polymer of polylysine. In this structure, the protenated imidazole rings of the histidine groups which do not interact with the nucleic acid because of the branch structure of histidylated polymer are able to react with the cellular membrane. the complexes between DNA and histidylated polylysine have a global surface charge which is slightly positive (+15 mV at neutral pH), (state of potential). This state of potential increases up to 40 mV when the pH is lowered to a pH of 5.5 due to imidazole protenation. This means that the imidazoles do not interact with the nucleic acid. Therefore, the combination of the prior art does not anticipate Applicants' invention and withdrawal of this ground of rejection is requested.

Claims 21 to 40 were rejected under the judicially created doctrine of obviousness type double patenting with respect to claims 9 to 15 of U.S. Patent No. 5,733,762 taken alone or in view of the Wang et al reference.

Applicants respectfully traverse this ground of rejection since it is not deemed that the U.S. Patent No. 5,733,762 claims the same invention as this. The said patent corresponds to the D1 reference cited by the Examiner and therefore, the arguments with respect to the lack of teachings of the D1 reference applies to this ground of rejection and therefore, a terminal disclaimer is not required. Therefore, withdrawal of this ground of rejection is requested.

In view of the amendments to the specification and claims and the above remarks, it is believed that the claims clearly point out Applicants' patentable contribution and favorable reconsideration of the application is requested.

Respectfully submitted,
Bierman, Muserlian and Lucas

By: 
Charles A. Muserlian #19,683
Attorney for Applicants
Tel.# (212) 661-8000

CAM:ds
Enclosures